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Extra Views

INT6

A Link Between the Proteasome and Tumorigenesis

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To reach the final state of malignancy, cancer cells must undergo a global transformation to acquire a wide range of novel activities.[1] For example, cancer cells must bypass all the signaling pathways that act to suppress uncontrolled cell division. They are immortal and refractory to apoptosis, which functions to eliminate aberrant cells. Cancer cells can evade detection by the immune system. They can migrate and establish colonies in numerous tissues and induce angiogenesis to maintain the supply of nutrients. Considering the fact that the spontaneous mutation rate in normal cells has been estimated to be just 10^{-7} per gene per cell division,[2] it is puzzling how cells can be reprogrammed so efficiently, within a relatively short period of time, to become cancerous.

The *INT6* gene was first identified from a screen in which the mouse mammary tumor virus (MMTV) was employed as an insertional mutagen to seek genes whose functions are critical for breast tumor formation.[3] MMTV insertion in mouse *INT6* appears to create C-terminally truncated proteins that are dominant-negative. Overexpression of these truncated proteins can transform cells in culture, and injection of these transformed cells into nude mice can induce tumor formation.[4,5] In human breast cancers, *INT6* expression is frequently diminished.[6-8] Intriguingly, *INT6* was isolated from a feral strain of mouse (Czech II), which has not been pre-selected for a high propensity for tumor formation and does not have any endogenous MMTV insertion into other *INT* genes.[3] These observations support a hypothesis that loss of *INT6* functions alone can have a profound impact on breast tumorigenesis. Despite the importance of *INT6* and breast cancer, the biological functions of *INT6* in humans remain open for investigation.

In a recent paper,[9] we uncovered a key conserved function of Int6 by characterizing its homolog, *yin6* (yeast *int6*), in the fission yeast *Schizosacchromyces pombe*. We demonstrated that Yin6 positively regulates the 26S proteasome, which functions to degrade polyubiquitinated proteins, by binding to and mediating the nuclear import and assembly of a proteasome regulatory subunit, Rpn5. As a result, the proteasome is inactivated in the *yin6* deletion (*yin6*Δ) mutant, causing accumulation of polyubiquitinated proteins, among which are mitotic cyclin (Cdc13) and securin (Cut2). Cyclin accumulation blocks cytokinesis and exit from mitosis, while securin accumulation impairs sister-chromatid separation. Thus, accumulation of these two proteins is at least partly responsible for the abnormally lengthy mitosis and inefficient chromosome segregation found in *yin6*Δ cells (see refs. 10 and 11; Fig. 1). We note that *yin6*Δ cells display additional phenotypes that are also present in proteasome mutants. For example, like *yin6*Δ cells,[10] nearly all known *S. pombe* proteasome mutants are resistant to microtubule depolymerizing drugs.[12] *yin6*Δ cells, as well as all the proteasome mutant cells examined (e.g., *mts2-3* and *pad1* mutants), bypass the requirement for nutrient starvation and mate efficiently in rich media (Fig. 2), suggesting that they are all defective in the cAMP signaling pathway. Because of these similarities in phenotypes, it is highly probable that all the abnormalities resulting from *yin6*Δ are due to proteasome inactivation.

Human and yeast Int6 are over 40% identical in protein sequence. As yeast Yin6 binds yeast Rpn5, so also human Int6 binds human Rpn5; most remarkably, human Int6 restores the yeast Rpn5 localization and rescues the growth defect in *yin6*Δ cells.[9-11,13] These results suggest that in humans, as in yeast, Int6 might regulate the proteasome, and that inactivation of human Int6 might lead to mitotic abnormalities and genetic instability due to cyclin and securin accumulation. Because abnormalities in the proteasome and mitotic fidelity can both impact broadly on multiple cell functions, this could explain why MMTV insertion in *INT6* alone seems sufficient to induce breast tumor formation in mice. Moreover, we created diploid *S. pombe* cells that lack either one or both copies of *yin6*. Interestingly, as shown in Figure 3, cells heterozygous for *yin6*Δ (*yin6*Δ/+) display a phenotype that is intermediate between wild type (+/+) and *yin6* null cells (*yin6*Δ/*yin6*Δ), indicating haploid insufficiency. If this also occurs in human cells, then the loss of just a single copy of *INT6* (or a reduction in its expression) may suffice to inhibit proteasome activities.

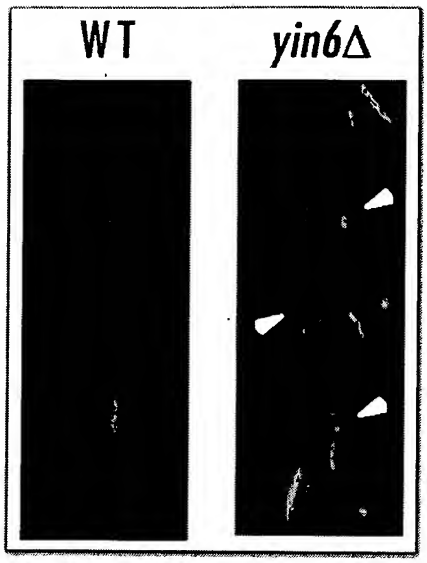

Figure 1. Accumulation of late mitotic cells. *yin6Δ* cells were pregrown at 30°C to early log phase, resuspended in fresh medium, and then shifted to either 30°C, as control, or to 20°C to grow for another 17 hr. The cells were fixed and stained as described.[10] The spindle and DNA staining were pseudo-colored green and red, and then merged. After the shift to 20°C, a disproportionally large number of mitotic *yin6Δ* cells were in late anaphase, as judged by the presence of a long spindle and post-anaphase arrays (arrowheads) in the same cell: 13% at 30°C and 50% at 20°C, as compared to ≤1.7 % in wild type cells at both 30 and 20°C.

Several lines of data suggest that Int6 preferentially regulates Rpn5. In our original two-hydrid screen, the only proteasome subunit that was isolated is Rpn5, and it was isolated multiple times.[9] Furthermore, Yin6 does not bind other proteasome subunits available for the two-hybrid system, Rpn9/Mts1, Rpn10/Pus1, Rpn11/Bts4, Rpt1/Mss1, Rpt2/Mts2, Rpt5/Sug2, or Rpt6/Sug1. Can Int6 interact and regulate other proteasome subunits? In an ongoing study, we have isolated Rpn7 as a high copy suppressor that rescues the phenotype of *yin6Δ* cells, and in plants, Int6 copurifies with Rpn7.[14] In humans, Int6 has been shown to bind Rpt4.[15] It is possible that, besides Rpn5, Yin6/Int6 may influence the function of Rpn7 and Rpt4. It is unclear why Rpn5 has not yet been identified in other systems as an Int6-binding protein. During the course of studying Rpn5 expression in *S. pombe*, we found that Rpn5 expression is highly regulated to maintain a low level in the cell (Yen et al., submitted). Moreover, Rpn5 has been identified as one of only 200 or so components that are expressed in common among many human stem cells.[16] Thus, we surmise that Rpn5 expression may be highly regulated in eukaryotes such that it is expressed at low levels or that it is expressed in certain cell types and/or during a particular process.

Human Int6 may also influence proteolysis indirectly via at least three proteins that have been identified as Int6-binding proteins, Tax,[17] RFP (Ring Finger Protein),[18] and p56.[19] Tax is an oncoprotein carried by Human T-cell Lymphoma Virus Type I. Tax binds and stimulates the activity of the proteasome.[20] RFP contains the RING finger, which is present widely among ubiquitin ligases (E3, reviewed in ref. 21), and localizes to the PML nuclear bodies, which are rich with the proteasome.[22] p56 was first identified as one of the molecules whose expression is induced by interferons, and it contains two ubiquitin-like domains (see the annotation in Genbank, Accession # Q15646), suggesting that it can also bind the proteasome.

In addition to the proteasome, Int6 also associates with the COP9 signalosome, CSN, which is best known for its role in plants to regulate photomorphogenesis.[23] In *S. pombe*, the CSN regulates the cell cycle check-point, while in flies, it is essential and regulates multiple developmental pathways. In cell culture, the CSN controls a wide range of signaling pathways, and may play important roles in tumorigenesis in humans. The biochemical functions of CSN have not been fully established, but, like Int6, it plays a role in regulating proteolysis. Components of the CSN frequently co-purify with certain proteasome components. Furthermore, a component in the CSN, CSN5, can activate E3 by removing Nedd8,[24] an ubiquitin-like molecule, which is conjugated to cullin to otherwise keep the cullin-associated E3 in the off-state. The proteasome regulatory complexes consist of the lid and base subcomplexes. Interestingly, the components in the lid and CSN are structurally similar, in that they all seem to contain the same number of subunits (eight) and the majority of them contain either the PCI or the MPN domain.[25] Because both the CSN and the proteasome lid are important for proteolysis and because their components are structurally similar, it has been hypothesized that either the entire CSN and the proteasome lid or some of their subunits may be interchangeable to degrade specific set of proteins. In plants, Int6 interacts with at least one of the CSN subunits, CSN7.[26] A *S. pombe* CSN7 homolog was also isolated in the same screen from which Rpn5 was identified as a Yin6 binding protein[9] (and our unpublished results). Since Yin6/Int6 also contains the PCI domain, it would be of interest to determine whether Yin6/Int6 plays a role in mediating an interaction between the CSN and the proteasome lid during proteolysis.

Int6 also co-purifies with the eIF3 complex in mammals, plants, and *S. pombe*, and thus was also named eIF3e.[11,13,27-29] The eIF3 is essential for initiating translation. Paradoxically, budding yeast,

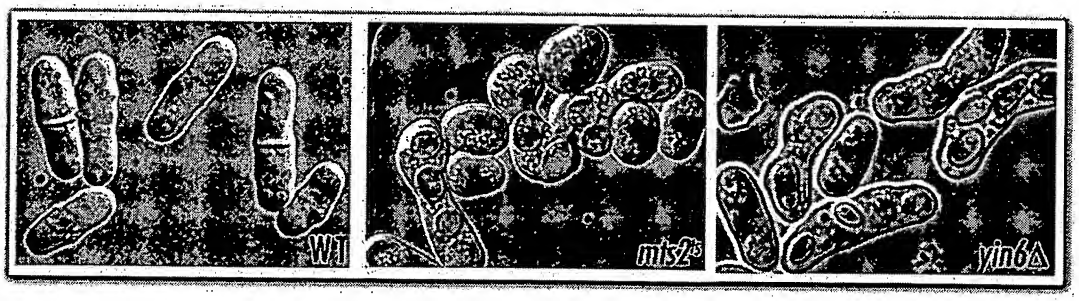

Figure 2. Mating without sufficient nutrient starvation. Various homothallic (*h90*) strains were patched on plates containing the rich medium (YEAU) and incubated at room temperature for 3 days. Note that some of the asci are abnormal and this may be caused by abnormal chromosome segregation during meiosis.[10,11,28]